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THE ACTION OF *N*-METHYL-*N*-NITROSOUREA ON NON-ESTABLISHED HUMAN CELL LINES IN VITRO. II. NON-RANDOM DISTRIBUTION OF CHROMATID ABERRATIONS IN DIPLOID AND DOWN'S CELLS

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Summary

Chromatid gaps, breaks and aberrations involved in interchanges induced by *N*-methyl-*N*-nitrosourea (MNU) were found non-randomly distributed on individual chromosomes and chromosome segments (G bands) both in human diploid fibroblasts and fibroblasts with trisomy 21 cultured in vitro. Aberration events were located exclusively in pale G bands. Considering cells in the first post-treatment mitosis, the pattern of aberration distribution, as revealed by the position of hot spots, varied with recovery time and was different in diploid and Down's cells. In comparison with diploid cells, the X chromosomes of Down's cells were not involved in aberrations. Despite the higher aberration frequencies of Down's cells, the number of hot spots and the proportion of aberrations located in hot spots were not increased in this cell type. Therefore, the increased chromosomal sensitivity to MNU of Down's cells does not reflect an increased sensitivity of special chromosomes or chromosome sites.

Introduction

Down's syndrome is a congenital disease characterized by trisomy 21 which predisposes the affected persons to the development of leukemia [19,25,28,49]. Furthermore, lymphocytes of patients with Down's syndrome are more sensitive than lymphocytes of healthy individuals to the action of X-rays [7,8,10, 21,42,43] and other chromosome-breaking agents [34,46]. Investigations on the clastogenic effect of *N*-methyl-*N*-nitrosourea (MNU) in diploid fibroblasts and fibroblasts with trisomy 21 in vitro [20] showed Down's cells to be more

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sensitive with respect to the induction of chromosomal aberrations and the inhibition of the progression of cells through the S phase of the cell cycle. These results verify the concept of a higher chromosomal sensitivity of Down's cells which is generally assumed to be due to differences in the repair capacity of diploid and Down's cells [18,43].

The association of an increased chromosomal sensitivity of Down's cells and the predisposition of patients with Down's syndrome to the development of tumours is of interest with respect to the hypothesis stating that somatic mutations play a causal role in the processes involved in transformation of normal cells to tumour cells. Assuming that special chromosome constitutions lead to malignant transformation of cells, as demonstrated by Rabinowitz and Sachs [2,38], it might be expected that the increased transformation frequency of Down's cells is a result of a more pronounced aberration clustering in chromosome regions important for malignant transformation.

There is good evidence of a non-random distribution of aberrations induced both by X-rays and chemical mutagens in chromosomes of plants, mammals and man [5,14,26,33,40,45,48]. Investigations of human chromosomes stained by the recently developed Q, G and R banding techniques also showed that induced aberrations were not distributed proportionally to chromosome length but clustered in special chromosome regions [4,6,16,30,44].

The present paper deals with the location of aberrations on chromosomes of diploid and Down's cells that were induced by the highly reactive mutagen [24,27] and carcinogen [12] MNU.

Attempts were made to answer the following questions. (1) Are MNU-induced aberrations non-randomly distributed in human chromosomes? (2) Are there chromosome sites which are preferentially involved in aberrations? (3) Is the pattern of aberration distribution dependent on the position of cells in the cell cycle during mutagen application? (4) Are there differences in the pattern of aberration distribution between diploid and Down's cells? (5) Are increased aberration frequencies of Down's cells due to an increased sensitivity of special chromosomes or chromosome segments to the chromosome-breaking effect of MNU?

Materials and methods

The cell culture technique used and the treatment procedure of cells with MNU have been described previously [20]. Embryonic diploid cells (46, XX) and cells with trisomy 21 (47, XX, +21), derived from the lung of deceased babies with Down's syndrome, were cultured in glass vessels at 37°C in Eagle's Minimal Essential Medium (MEM) supplemented with 20% inactivated calf serum (inoculation density, 10^5 cells per ml). In the exponential phase of growth, cells were incubated for 60 min in the dark with medium containing MNU. (Final concentration of MNU: 1×10^{-3} M; pH 6,8; 37°C.) After MNU treatment the cell layer was rinsed twice with phosphate-buffered saline (PBS), and conditioned medium was added.

Chromosomes were prepared from trypsinized colchicine-arrested cells (final concentration of colchicine: 0.0001%; 4 h) after recovery times of 12, 24 and 42 h by routine techniques (hypotonic treatment with 0.9% sodium citrate,

fixation of cells in acetic acid—methanol, 1 : 3, spreading of chromosomes on wet slides). Air-dried slides were incubated for 12—48 h at a relative humidity of 40—50% (40°C).

For Giemsa staining of chromosomes a modified G-banding technique was used involving the following steps: (1) Incubation of slides in 0.025 M phosphate buffer (pH 6.8), 45 min, 60°C. (2) Rinsing in cold phosphate buffer. (3) Staining 4-5 min in Giemsa-trypsin solution: 20 ml 0.025 M phosphate buffer, 6 ml 0.1% trypsin (Difco) in buffer, 0.6 ml Giemsa solution. Metaphases with aberrant chromosomes were photographed on DK5 film (ORWO) through a VG9 filter. Karyotyping and classification of chromosomal aberrations were done in conformity with the recommendations given at the Paris Conference [35]. Aberrations were classified as chromatid gaps, chromatid breaks and chromatid interchanges (see Fig. 1).

Results

Location of aberrations

The locations of chromatid breaks, chromatid gaps and chromatid interchanges, the most frequent aberration types induced by MNU in the first mitosis after treatment, are shown in Fig. 1. Gaps, breaks and interchange events have always been found to be located in pale G bands. In those cases in which dark bands had been deleted the breaks were found adjacent to the missing dark band (Fig. 1B). Such chromatid deletions were observed only rarely.

Fig. 2 shows the positions of 776 aberrations induced by 1 mM MNU in chromosomes of diploid and Down's cells after recovery times of 12, 24 and 42 h. As reported elsewhere [20], aberration frequencies (breakage events per cell) were 0.168, 0.172 and 0.264 in diploid cells, and 0.242, 0.453 and 0.515 in Down's cells. Cells collected at the recovery times used were in late S (12 h recovery), early S (24 h recovery), and G_1 (42 h recovery), respectively, during treatment with MNU.

Interchromosomal distribution of aberrations

A comparison of the number of aberrations expected on the basis of their length-proportional distribution with the number of observed aberrations per chromosome (Table I) shows that, especially after a short recovery time, aberrations are non-randomly distributed over the chromosomes (χ^2; GF 21; $P < 0.01$). The significant deviation from a random distribution of aberrations was due to clustering of aberrations in certain chromosomes, as revealed by a comparison of expected and observed frequencies of involvement of individual chromosomes in aberrations ($3.38 < \chi^2 < 38.35$; GF 1; $0.07 < P < 0.01$): in diploid cells chromosomes 2 and 9 (recovery time, 12 h), and in Down's cells chromosomes 1 and 5 (recovery time: 12 h) and chromosome 4 (24 h recovery) were preferentially involved in aberrations. This observation shows that the interchromosomal distribution of aberrations may vary with respect to cell type and recovery time. The fluctuation of the involvement of individual chromosomes in aberrations is evident from Table I. The complete lack of aberrations involving the X chromosomes of Down's cells and chromosomes 21 of both cell lines is noteworthy. The involvement of chromosomes in chromatid

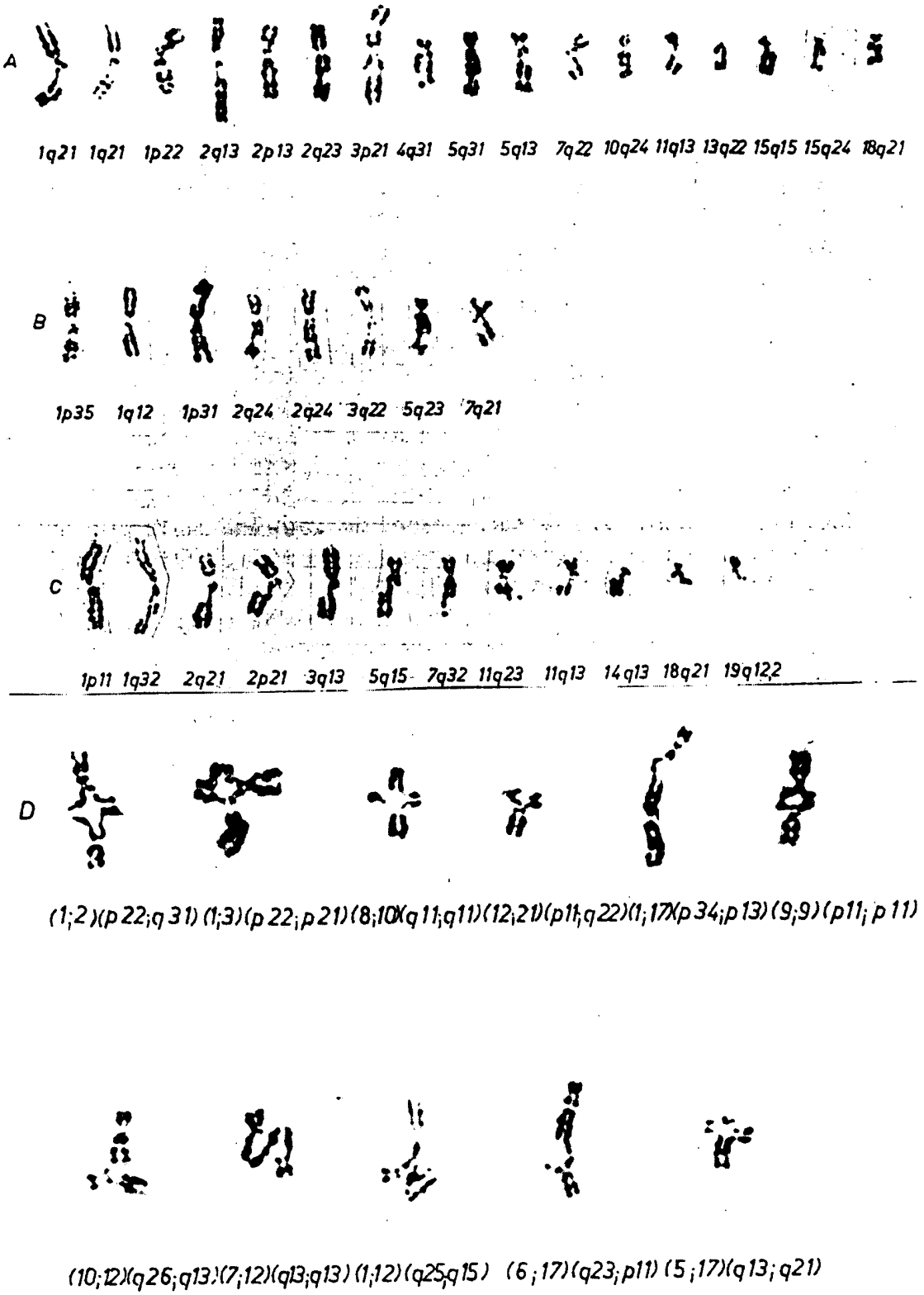

Fig. 1. Types of aberration and their locations in G banded chromosomes. (A) chromatid gaps; (B) chromatid gaps accompanied by loss of one or two dark bands; (C) chromatid breaks; (D) chromatid interchanges. Numbers at the bottom of each chromosome designate the aberration locus.

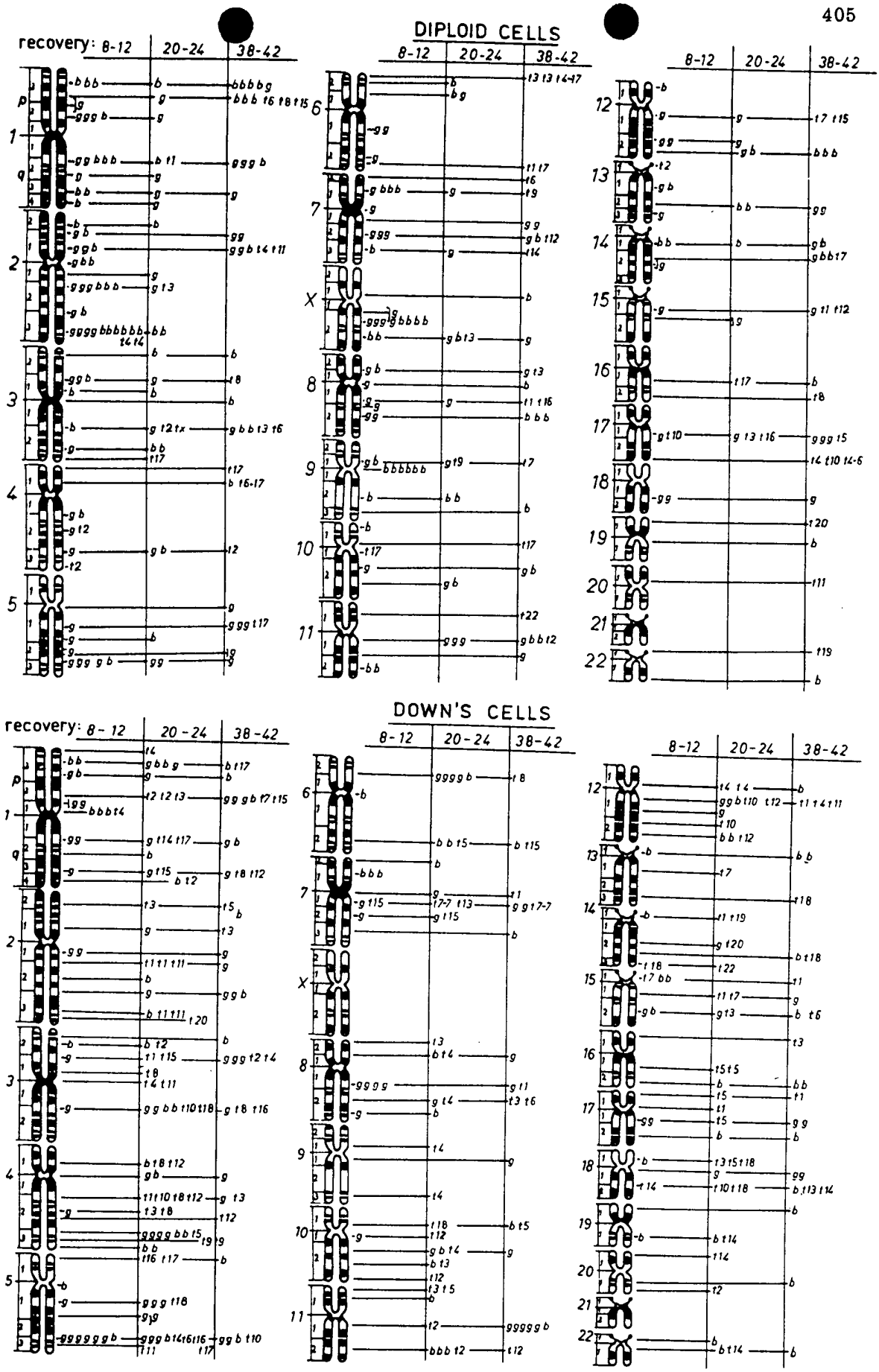

Fig. 2. Location of MNU-induced aberrations in chromosomes of diploid and Down's cells after recovery times of 12, 24 and 42 h. In the schematic representation of chromosomes, dark bands divided by thin lines tend to fuse. g, chromatid gaps; b, chromatid breaks; t2, chromatid translocation involving chromosome 2, respectively.

TABLE I

OBSERVED (A) AND EXPECTED (B) ABERRATION FREQUENCIES AFTER RECOVERY TIMES OF 12, 24 AND 42 H IN DIPLOID AND DOWN'S CELLS. AVERAGE VALUES ($\bar{x}$) OF RELATIVE CHROMOSOME LENGTH AND THEIR STANDARD DEVIATION ($s_{\bar{x}}$) BASED ON MEASUREMENTS OF 20 KARYOGRAMS

Chromosome number	Rel. chromosome length		Diploid cells						Down's cells					
			12		24		42		12		24		42	
	$\bar{x}$	$s_{\bar{x}}$	A	B	A	B	A	B	A	B	A	B	A	B
1	9.25	0.29	17	12.4	8	5.4	16	10.2	12	4.9	19	15.2	16	9.0
2	8.40	0.26	32	11.3	6	4.9	7	9.2	2	4.5	12	13.8	8	8.1
3	7.05	0.30	6	9.5	9	4.1	11	7.8	3	3.7	13	11.6	9	6.8
4	6.27	0.24	6	8.4	2	3.6	4	6.9	1	3.3	21	10.3	4	6.1
5	6.23	0.21	8	8.4	3	3.6	7	6.9	9	3.3	17	10.2	7	6.0
6	5.92	0.16	3	9.7	3	3.4	5	6.5	1	3.1	8	9.7	3	5.7
7	5.38	0.15	9	7.2	2	3.1	8	5.9	6	2.9	8	8.8	6	5.2
X	4.96	0.15	10	6.7	3	2.9	2	5.5	—	2.6	—	8.1	—	4.8
8	4.92	0.15	7	6.6	1	2.9	8	5.4	5	2.6	8	8.1	5	4.8
9	4.57	0.16	14	6.1	4	2.7	2	5.0	—	2.4	2	7.5	1	4.4
10	4.49	0.13	3	6.0	2	2.6	3	4.9	1	2.4	8	7.4	3	4.4
11	4.41	0.14	2	5.9	3	2.6	6	4.9	—	2.3	8	7.2	7	4.3
12	4.37	0.14	4	4.9	4	2.5	5	4.8	—	2.3	12	7.2	4	4.2
13	3.72	0.12	4	5.0	2	2.2	2	4.1	1	2.0	1	6.1	3	3.6
14	3.25	0.11	4	4.7	1	2.0	6	3.9	2	1.9	5	5.8	2	3.4
15	3.52	0.11	1	4.7	1	2.0	3	3.9	5	1.9	4	5.8	4	3.4
16	3.06	0.10	—	4.1	1	1.8	2	3.4	—	1.6	3	5.0	3	3.0
17	2.94	0.09	2	3.9	3	1.7	7	3.2	2	1.6	4	4.8	4	2.7
18	2.79	0.09	2	3.6	—	1.6	1	3.1	2	1.5	6	4.6	5	2.2
19	2.25	0.08	—	3.0	—	1.3	2	2.5	1	1.2	2	3.7	2	2.2
20	2.25	0.08	—	3.0	—	1.3	1	2.5	—	1.2	2	3.7	2	2.2
21	1.66	0.07	—	2.2	—	1.0	—	1.8	—	0.9	—	2.7	—	1.6
22	1.94	0.07	—	2.6	—	1.1	2	2.1	—	1.0	3	3.2	1	1.9
χ^2			81.76		21.04		24.46		51.65		44.10		14.64	
P			<0.001		0.5		0.3		<0.001		<0.01		0.3	

TABLE II

POSITION OF "HOT SPOTS" (G BANDS WITH ABERRATION FREQUENCIES HIGHER THAN THE UPPER VALUE OF THE CONFIDENCE INTERVAL) AFTER DIFFERENT RECOVERY TIMES IN CHROMOSOMES OF DIPLOID AND DOWN'S CELLS

Numbers in parentheses: observed aberration frequencies (in %). The expected aberration frequency always amounts to 1.09%.

	Diploid cells				Down's cells			
Recovery time (h)	12	24	42	Total	12	24	42	Total
	2q21(5.3) 2q33(9.0) Xq22(6.0) 9p13(9.8)	—	1p32(6.0) 3q21(7.0)	1p34(3.1) 1q21(3.8) 2q21(3.1) 2q33(4.9) 3q21(3.8) 5q31(3.1) 9p13(5.6) 17q21(3.1)	1p12(10.9) 5q31(15.2)	4q31(4.9) 5q31(5.6)	11q13(8.0)	3q21(3.8) 5q31(7.6) 7q11(3.4)

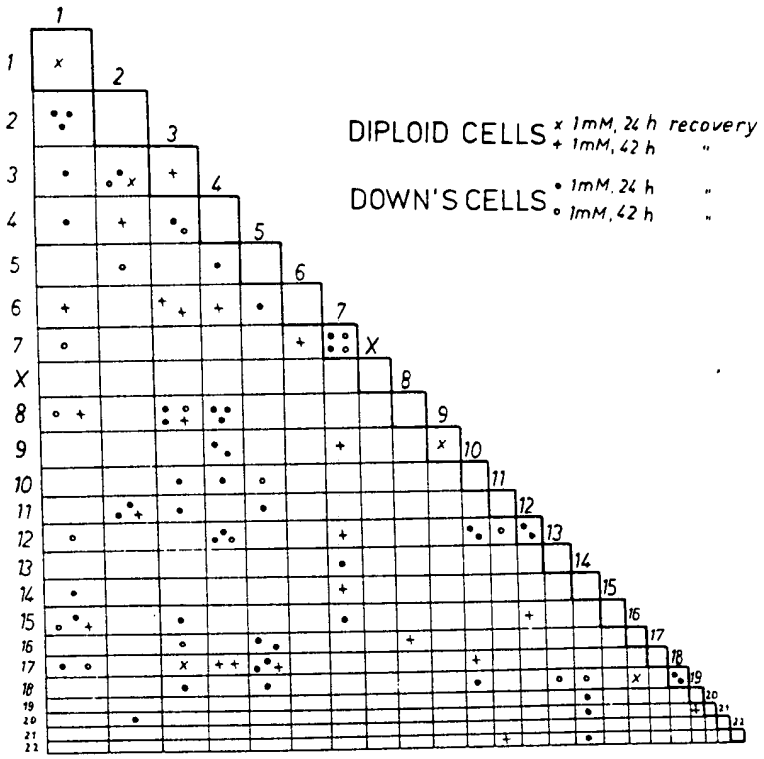

Fig. 3. Representation of the involvement of individual chromosomes in chromatid interchanges.

interchanges is demonstrated in Fig. 3. From the distribution of marks a non-random involvement of chromosomes in interchanges may be deduced. However, owing to the low number of interchanges, a statistical analysis was impossible.

Intrachromosomal distribution of aberrations

As mentioned above, aberrations were exclusively located in G^- bands. Assuming that all G^- bands were involved with the same probability in aberrations, the expected aberration frequency per G^- band should be 1.09% of the total number of aberrations (92 G^- bands were considered which have no tendency to disappear by fusion of adjacent G^+ bands). A comparison of the expected aberration frequencies with the number of observed aberrations revealed a non-random distribution of aberrations in G^- bands for all recovery times tested. (Diploid cells: 12 h recovery, $\chi^2 = 320.53$; 24 h recovery, $\chi^2 = 118.70$; 42 h recovery, $\chi^2 = 213.63$. Down's cells: 12 h recovery, $\chi^2 = 194.40$; 24 h recovery, $\chi^2 = 173.17$; 42 h recovery, $\chi^2 = 178.13$; GF 91, $P < 0.01$.)

Confidence limits * of the expected number of aberrations per G^- band showed some G^- bands to be significantly more frequently involved in aberrations than expected (Table II). With respect to the position of these bands

* Confidence limits were established from tables for percentage confidence limits ($\alpha = 0.05$) by Bruhnke [50]. These values satisfactorily correspond to confidence limits (Z) based on the known expression $Z = k \pm [½ + \mu_{\alpha/2} \sqrt{k\,(1 - k/N)}]$, wherein k = expected frequency of aberrations per segment and N = number of aberrant cells ($\alpha = 0.01$). The equation is based on an approximation of the binominal distribution to a normal distribution.

(which might be called hot spots), the following conclusions may be drawn. (1) The position of hot spots varies with recovery time. (2) Frequently appearing hot spots are 3q21 and 5q31 in both diploid and Down's cells. However, the position of most hot spots is different in the two cell types. (3) The number of hot spots in Down's cells is lower than in diploid cells.

Discussion

Possible reasons for a non-random aberration distribution

A non-random distribution of aberrations may be explained in two ways: it might be a consequence of a non-homogeneity of the structure of chromosomes (concerning, e.g. clustering of bases, differences in the secondary structure of DNA and/or in the arrangement of chromatin fibers in the nucleus during interphase) or an expression of functional diversity. With respect to the second possibility, the asynchrony of DNA replication of different chromosomal regions during the S period of the cell cycle is suggested to be a causal factor in the establishment of a non-random pattern of aberration distribution, especially if alkylating agents are being used for aberration induction. This is concluded from the following facts: It is well known that the clastogenic effect of alkylating agents is bound to the replication of DNA during the S period [13]. Therefore, primary lesions due to alkylations (e.g. alkylated bases and phosphate groups) are expected to be transformed into pre-mutational lesions during DNA replication. Since DNA lesions due to alkylation have become removed from DNA in dependence on time available for repair [37,47], their amount in the replication fork of DNA is expected to be a function of the time between the induction of primary lesions in DNA and the replication of DNA. If, e.g. the induction of primary lesions occurs in the pre-DNA-synthetic phase, aberrations are expected to be more frequently located in chromosome regions containing early replicating DNA than in those with later replicating DNA. DNA regions may, however, differ in their involvement in primary lesions and efficiency of repair, respectively, which makes the situation even more complicated.

Variations in the pattern of aberration distribution

Variations in the pattern of aberration distribution in dependence on the position of cells in the cell cycle during mutagen treatment seem to be a neccessary consequence of the above considerations regarding the asynchrony of DNA replication in chromosomes. Furthermore, in cells treated during different parts of the S phase with alkylating agents, variations may be caused directly by the differential replication pattern of chromosomes. Chromosome regions that have finished DNA replication before treatment with the alkylating agent are not expected to become involved in aberrations visible during the same cell cycle. Aberrations would then be expected to be arranged in more distinct clusters after short recovery times as compared with longer ones. This is borne out by the experimental data, since after a recovery time of 12 h the difference between observed and expected aberration frequencies (based on the assumption of a random distribution of aberrations) is larger than that found after a recovery time of 24 h.

Location of aberrations in G^- bands

Caspersson et al. [6] showed X-ray-induced aberrations in banded human chromosomes to be preferentially located in Q^- bands. The bulk of additional experimental data obtained on banded mammalian chromosomes confirmed this observation. They definitely indicate a preferential, if not exclusive, location of aberrations induced both by X-rays [15,22,41,44] and chemical mutagens [22,30,36,39] in Q^-, G^- or R^+ bands. Similar results have been obtained for the breakage points of spontaneous translocations in chromosomes of the mouse [11] and man [51]. Only few publications report on a random distribution of aberrations between pale and dark bands [4,17]; a preferential involvement of G^+, Q^+, or R^- bands in aberrations has never been found.

It may be argued that the precise localization of aberrations with respect to pale and dark G bands in human chromosomes is limited for technical reasons. However, if e.g. chromatid breaks were in fact located in G^+ bands, aberration figures as demonstrated in Fig. 4b would be expected to occur. Such aberrations have never been found in the present investigation. It is therefore concluded that MNU-induced breakage events are exclusively located in G^- bands.

G^+ bands have shown all properties of constitutive heterochromatin [1,3,9, 51]. It is therefore likely that G^- and R^+ bands, in which aberrations are preferentially, if not exclusively, located, correspond to euchromatin or facultative heterochromatin.

Sometimes (e.g. on chromosome 9 of diploid cells) aberrations are clustered within the centromere. Since the regions adjacent to the centromere have been demonstrated to be C^+, it may be argued that the aberrations are located in the pericentric heterochromatin. A detailed localization of the aberrations, however, unequivocally placed them in the centromere proper.

The finding of the location of aberrations in euchromatin of human chromosomes seems to be in contradiction of the concept developed from the analysis of aberration distribution in chromosomes having large clusters of constitutive heterochromatin such as the X chromosomes of *Microtus agrestis* [31,32] or the chromosomes of *Vicia faba* [40,45]. According to this concept, aberrations are preferentially located in chromosome regions rich in heterochromatin. This discrepancy may be explained by assuming that the preferential location of aberrations in chromosome regions rich in heterochromatin is not due to a clustering of aberrations in heterochromatin itself, but the result of an increased tendency of euchromatin adjacent to it to be involved in aberrations. If aberrations are indeed restricted to euchromatin, a protection mechanism for aberrations induction in heterochromatin is to be assumed, or its particular

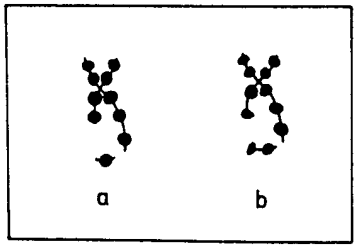

Fig. 4. Schematic representation of the banding pattern of a chromosome expected for a break in a pale (a) or dark (b) band.

structure is incompatible with processes leading to the manifestation of chromosomal aberrations.

Is the increased chromosomal sensitivity of Down's cells associated with an increased clustering of aberrations?

If this were so, both the number of G^- bands showing up as hot spots and the proportion of aberrations located in hot spots, respectively, would be expected to be higher in Down's cells than in diploid cells. However, the results reported here show that, although Down's cells are nearly twice as sensitive as diploid cells (in average 0.403 breakage events per cell in comparison with 0.201), the number of hot spots and the proportion of aberrations located in hot spots is lower than in diploid cells. It may thus be concluded that the increased aberration frequencies of Down's cells induced by MNU do not reflect a more pronounced aberration clustering in this cell type. A frequently discussed question is whether or not aberrations of special chromosomes may be the starting point in the transformation of normal cells to tumour cells. There are some observations claimed to be in favor of this hypothesis. (1) Chromosome variation in many types of tumour is non-random [23,29]. (2) Individuals with chromosmal disorders and patients suffering from the so-called chromosome breakage syndromes are predisposed to the development of tumors [15]. (3) Loss from or addition to the normal karyotype of special chromosomes induced by carcinogenic substances may cause an expression of malignant properties of cells [2,38]. From the above-mentioned hypothesis it may be expected that the increased sensitivity to the clastogenic action of carcinogens of cells predisposed to malignant transformation, such as Down's cells, is accompanied by an increased clustering of aberrations in special chromosomes which might be important in malignant transformation. The results reported here do not support this conclusion.

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References

1 Bahr, F., The ultrastructure of chromosomes, in T. Caspersson and L. Zech (eds.), Chromosome Identification-techniques and Application in Biology and Medicine, Nobel Symposium 23, Academic Press, New York and London, 1972.

2 Bloch-Shtacher, N. and L. Sachs, Chromosome balance and the control of malignancy, J. Cell Physiol., 87 (1976) 89—100.

3 Bostock, C.J., D.M. Prescott and F.T. Hatch, Timing of replication of the satellite and main band DNAs in cells of the Kangaroo-rat (Dipodomys orddi), Exptl. Cell Res., 74 (1972) 487—495.

4 Bourgois, C.A., Distribution of mitomycin C-induced damage in human chromosomes with special reference to regions of repetitive DNA, Chromosoma, 48 (1974) 203—211.

5 Brogger, A., Chromosome damage in human cells induced by hydroxylamine, Hereditas, 69 (1971) 19—24.

6 Caspersson, T., U. Haglund, B. Lindell and L. Zech, Radiation induced non-random chromosome breakage, Exptl. Cell Res., 75 (1972) 541—543.

7 Chudina, A.P., Issledowanie radiochustvitelnosti kromosom chelvyeka v norme i bolezui Downa, Genetika, 6 (1968) 99—110.

8 Chudina, A.P., T.C. Malyutina and E.E. Pogosyane, Sravenemye radiochustvitelnosti kromosom v kultiviruyemike leukocitaki perifichetloy v norme i sindrome Downa, Genetika, 4 (1966) 51—63.

9 Comings, D.E., Methylation of euchromatic and heterochromatic DNA, Exptl. Cell Res., 74 (1972) 383—390.

10 Debakan, A.S., R. Thron and J.K. Streusing, Chromosmal aberrations in irradiated blood and blood cultures of normal subjects and of selected patients with chromosomal abnormality, Radiat. Res., 27 (1966) 50—63.

11 De Boer, P. and M. van Gijsen, The location of the position of the breakpoints involved in the T 26 H and T 70 H mouse translocations with the aid of Giemsa-banding, Can. J. Genet. Cytol., 16 (1974) 783—788.

12 Druckrey, H., R. Preussmann, D. Ivankovic and D. Schmahl, Organotrope carcinogene Wirkungen bei 65 verschiedenen *N*-Nitroso-Verbindungen an BD-Ratten, Zeitschr. f. Krebsforschg., 69 (1967) 103—201.

13 Evans, H. and D. Scott, The induction of chromosome aberrations by nitrogen mustard and its dependence on DNA synthesis, Proc. Roy, Soc. B., 173 (1969) 491—512.

14 Gebhart, E., Aberrationsverteilung nach Einwirkung von Myleran auf menschliche Chromosomen in vitro, Chromosoma, 29 (1970) 336—348.

15 German, J., Chromosomal breakage syndromes, Birth Defects, 5 (1969) 117—130.

16 Holmberg, M. and J. Jonasson, Preferential location of X-ray induced chromosome breakage in the R-bands of human chromosoms, Hereditas, 74 (1973) 57—67.

17 Holmberg, M. and J. Jonasson, Synergistic effect of X-ray and UV irradiation on the frequency of chromosome breakage in human lymphocytes, Mutation Res., 23 (1974) 213—221.

18 Horikawa, M., M. Watanabe and T. Sugahara, Changes in sensitivity to radiations and a chemical carcinogen during cell cycle and their relation to DNA damage, Studia Biophysica, 53 (1975) 5—7.

19 Jackson, E.W., J.H. Turner, M.R. Klauber and F.D. Norris, Down's syndrome: variation of leukemia occurrence in institutionalized populations, J. Chron. Dis., 21 (1968) 247—253.

20 Kaina, B., H. Waller, M. Waller and R. Rieger, The action of *N*-methyl-*N*-nitrosourea on non-established human cell lines in vitro. I. Cell cycle inhibition and aberration induction in diploid and Down's fibroblasts, Mutation Res., 43 (1977) 00—000.

21 Kucerova, M., Comparison of radiation effects in vitro upon chromosomes of human subjects, Acta Radiol., 6 (1967) 441—448.

22 Kucerova, M. and Z. Polivkova, Banding technique used for the deletion of chromosomal aberrations induced by radiation and alkylating agents TEPA and epichlorohydrin, Mutation Res., 34 (1976) 279—290.

23 Levan, G. and F. Mitelman, Clustering of aberrations to specific chromosomes in human neoplasms, Hereditas, 79 (1975) 156—160.

24 Loveless, A. and L.C. Hampton, Inactivation and mutation of coliphage T 2 by *N*-methyl- and *N*-ethyl-*N*-nitrosourea, Mutation Res., 7 (1969) 1—12.

25 Merili, D.H. and J.S. Harris, Mongolism and acute leukemia; report of 4 cases, J., Dis. Child., 92 (1956) 41—44.

26 Michaelis, A. and R. Rieger, On the distribution between chromosomes of chemically induced chromatid aberrations. Studies with a new karyotype of *Vicia faba*, Mutation Res., 6 (1968) 81—92.

27 Michaelis, A., J. Schöneich and R. Rieger, Chromosomenaberrationen bei *Vicia faba* und Ascitestumoren der Maus nach Einwirkung von *N*-Nitroso-*N*-Methylharnstoff, Chromosoma, 16 (1965) 101—123.

28 Miller, R.W. and J.F., Fraumeni, Down's syndrome and neonatal leukemia, Lancet, 11 (1968) 404.

29 Mitelman, F., J. Mark, G. Levan and A. Levan, Tumour etiology and chromosome patterns, Science, 176 (1972) 1340—1341.

30 Morad, M., J. Jonasson and J. Lindsten, Distribution of mitomycin C induced breaks on human chromosomes, Hereditas, 74 (1973) 273—282.

31 Natarajan, A.T. and G. Ahnström, Induced chromosomal aberrations and heterochromatin, in Modern Aspects of Cytogenetics. Constitutive Heterochromatin in Man, Symp. Med. Hoechst 6, Schattauer Verlag, Stuttgart, New York, 1973, p. 215—223.

32 Natarajan, A.T., G. Ahnström and R.P. Sharma, Heterochromatin and chromosomal aberration in *Microtus agrestis*: role of chromosomal association, Mutation Res., 22 (1974) 73—79.

33 Obe, G., Die Verteilung Miracil D induzierter achromatischer Läsionen und Chromatidbrüche auf den Chromosomen menschlicher Leukozyten, Z. Naturforsch., 265 (1970) 115—116.

34 O'Brien, R., L.P. Poon, E. Kline and J.W. Parker, Susceptibility of chromosomes of patients with Down's syndrome to 7,12-dimethylbenz(a)anthracene-induced aberrations in vitro, Intern. J. Cancer, 8 (1971) 202—210.

35 Paris Conference (1971), Standardization in Human Cytogenetics, Birth Defects, Original article series, Vol. 8, No. 7 (1972).
36 Pathak, S., M.Mc. Gill and T.C. Hsu, Actinomycin D effects on mitosis and chromosomes: sticky chromatides and localized lesions, Chromosoma, 50 (1975) 79—88.
37 Plant, J.E. and J.J. Roberts, A novel mechanism for the inhibition of DNA synthesis following methylation: the effect of *N*-methyl-*N*-nitrosourea on HeLa cells, Chem.-Biol. Interactions, 3 (1971) 337—342.
38 Rabinowitz, Z. and L. Sachs, Control of reversion of properties in transformed cells, Nature, 225 (1970) 136—139.
39 Reeves, B.R. and C. Margoles, Preferential location of chlorambucil-induced breakage in the chromosome of normal human lymphocytes, Mutation Res., 26 (1974) 205—208.
40 Rieger, R., A. Michaelis, I. Schubert, P. Döbel and H.W. Jank, Non-random intrachromosomal distribution of chromatid aberrations induced by X-rays, alkylating agents and ethanol in *Vicia faba*, Mutation Res., 27 (1975) 69—79.
41 San Roman, C. and M. Bobrow, The sites of radiation induced breakage in human lymphocytes chromosomes, determined by quinacrine fluorescence, Mutation Res., 18 (1973) 325—331.
42 Sasaki, M.S. and A. Tonomura, Chromosomal radiosensitivity in Down's syndrome, Jap. Jour. Human Genet., 14 (1969) 81—92.
43 Sasaki, M.S., A. Tonomura and S. Matsubara, Chromosome constitution and its bearing on the chromosomal radiosensitivity in man, Mutation Res., 10 (1970) 617—633.
44 Seabright, M.A., High resolution studies on the pattern of induced exchanges in the human genome, Chromosoma, 40 (1973) 333—346.
45 Schubert, I. and R. Rieger, Non-random intrachromosomal distribution of radiation-induced chromatid aberrations in *Vicia faba*, Mutation Res., 35 (1976) 79—90.
46 Schuler, D., G. Fekete and M. Dobos, Mit einem alkylierenden Agens (Zitostop) in vitro induzierte Mutationen bei Malignomen und bei Syndromen, die zur Malignität disponieren, Humangenetik, 16 (1972) 329—336.
47 Scudiero, D. and B. Strauss, Accumulation of single-stranded regions in DNA and the block to replication in a human cell line alkylated with methyl methane sulfonate, J. Mol. Biol., 83 (1974) 17—34.
48 Stopik, D., Studie zur intrachromosomalen Bruchverteilung bei menschlichen Leukozyten, Humangenetik, 14 (1971) 24—32.
49 Wald, M., W.H. Borges, C.C. Li, J.H. Turner and M.C. Harnois, Leukaemia associated with mongolism, Lancet, 1 (1961) 1228.
50 Weber, E., Grundriss der biologischen Statistik, 4. Auflage., VEB Gustav Fischer Verlag Jena, 1961.
51 Weisblum, B. and P.L. De Haseth, Quinacrine, a chromosome stain specific for deoxyadenylate-deoxythymidylate-rich regions in DNA, Proc. Natl. Acad. Sci. USA, 69 (1972) 629—632.
52 Welch, J.P. and C.L.Y. Lee, Non-random occurrence of 7—14 translocations in human lymphocytes cultures, Nature, 255 (1975) 241.